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Mate recognition and antennal morphology of *Octodonta nipae* (Coleoptera: Chrysomelidae) adults

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ABSTRACT

The nipa palm hispid beetle, *Octodonta nipae* (Maulik) has been killing palm trees since its introduction into Hainan province, China, from Malaysia in 2001. It continues to spread within Hainan province, northeast to Fujian province, and northwest to Yunnan province within China. Knowledge on signals involved in mate location and recognition could help develop effective integrated pest management programs. In the present study, we first experimentally proved that antennae were essential in success of *O. nipae* mating. We then excised various segments/flagellomeres of adult male and female antennae and observed their mating behavior. Results revealed that the 5th to 9th flagellomeres, especially those of males, were important for the mating success. Finally, in an attempt to elucidate the types of antennal sensilla accountable for the mating success, morphology of *O. nipae* antennae was studied in detail with scanning electron microscopy. Six types of sensilla were distinguished: aporous sensilla trichodea (T1), multiporous sensilla trichodea (T2), aporous sensilla chaetica (Ch1), uniporous sensilla chaetica (Ch2), multiporous sensilla basiconica (B), and Böhm sensilla (Bm). Aporous sensilla trichodea is the most abundant; multiporous sensilla trichodea and sensilla basiconica are considered as olfactory receptors, and uniporous sensilla chaetica as gustatory receptor. Importance of flagellomeres 5–9 in mating success seemed to correspond to the abundance of sensilla on these segments.

Introduction

The nipa palm hispid beetle, *Octodonta nipae* (Maulik) (Coleoptera: Chrysomelidae) is native to Malaysia and has been a serious pest to palm trees (Family Arecaceae) in Hainan province, China since its accidental introduction in 2001. Larvae and adults mainly feed on young leaves, which consequently causes young stems to shrink, curl, or die (Hou et al., 2011; Steiner, 2001; Sun et al., 2003; Zhang, 2003). Besides spreading within Hainan province, it has recently spread to Yunnan, and Fujian provinces, and is causing significant economic, ecological, and environmental losses in these areas (Zhang et al., 2015). Since *O. nipae* feeds within the leaves of palm, chemical control with pesticides is ineffective (Hou et al., 2014a, 2014b; Hou and Weng, 2010; Tang et al., 2014b; Xu et al., 2011). Chemical control is neither desirable for controlling urban pests such as *O. nipae* due to human health concerns. The long life history, high adaptability and reproductive capacity further make eradication of *O. nipae* difficult and challenging (Feng and Hou, 2015; Hua et al., 2014; Li et al., 2014; Tang et al., 2014a).

For reproduction to occur in non-parthenogenetic insects, sexually mature males and females need to locate each other (Gullan and Cranston, 2010). Multimodal signals (e.g., chemical, visual, audial, and tactual information) might be used to locate and recognize mates. The compound eyes are the most important visual organs for many insects. Antennae, on the contrary, may be involved in olfaction, mechanoreception, gustation, thermo-, and hygro-reception (Allison et al., 2004; Hanks, 1999; Iwabuchi, 1985; Schneider and Seibt, 1969; Sen and Mitchell, 2001). Antennal sensilla are broadly grouped into aporous, uniporous and multiporous sensilla based on morphological differences (Zacharuk, 1985). Different antennal sensilla play different roles in feeding, mating, roosting, defense and migration (Na et al., 2008; Paczkowski et al., 2011), like bed bug *Cimex lectularius* (Olson et al., 2014) with two types of sensillar, may involve in alarm, aggregation, and host-finding behaviors; brown spruce longhorn beetle *Tetropium fuscum* (MacKay et al., 2014) with sensilla chaetica play a role in mate recognition and feeding.

The morphology and functions of antennal sensilla in mating recognition of *O. nipae* remain unknown. In this study, we first investigated role of compound eyes in mating behavior of *O. nipae*.

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Secondly, we studied role of different flagellomeres in mating by removing various segments. Because different flagellomeres were found to be differentially involved in mating, the morphology (i.e., antennal sensilla) of the whole antennae were further studied by using scanning electron microscopy.

Material and methods

Insect

Adult males and females used in the study were collected from a colony maintained in the laboratory for several generations in a controlled environment (25 ± 1 °C, 70 ± 5% RH, 12 L: 12D). They were reared on young leaves of *Phoenix canariensis* Hort EX Chabaud (natural host plant of *O. nipae*) in sterile transparent plastic bottles (diameter: 70 mm; height: 105 mm; Jiafeng Horticultural Products Co. Ltd., Shanghai, China) with ventilated lids. The male and female were kept separately upon emergence to avoid mating before experimentation.

Observation of mating behavior

Because mating behavior of *O. nipae* occurs both at the light and dark phase (Zhang, 2015), to simplify the experiment, observations of mating behavior were conducted during the day time. One hundred sexually mature males (15 d after emergence) were each randomly paired with one mature female of the same age. Each pair was placed in a glass culture dish (diameter: 50 mm; height: 10 mm; Jiafeng Horticultural Products Co. Ltd., Shanghai, China) containing one freshly cut young *P. canariensis* leaf (25–40 mm long) maintained at 25 ± 1 °C and 70 ± 5% RH. The mating behavior of each pair was monitored in the light by a 30× high speed auto focus DSP color CCD camera (1/4 in. SONY Super HAD CCD; Sony Corp. Japan) for 2 h or upon the completion of the first copulation (ca. 80% copulation occurred in 2 h).

Role of visual cue in mating

To elucidate role of visual cues in mating, mating behavior of *O. nipae* in the day time (from 8 AM to 10 AM) was recorded with two treatments: one in the light and another in the dark. The experimental procedure and instruments followed those described in the preceding experiment, except that the camera that was used to record the pairs in the dark was equipped with four infrared lamps (Model: F96; Veian Co. Ltd., Guangzhou, China). The experiment was a blocked (i.e., time) design with seven pairs per treatment being monitoring at the same time. The experiment was repeated at five different times (blocks) which resulted in 35 pairs per treatment.

Role of antenna in mating

The experiment was designed to test the function of the entire antennae in the mating in the dark. This experiment had four treatments: (1) one male with both antennae excised (i.e., antenna-excised male) and one intact female; (2) one intact male and one female with both antennae excised (i.e., antenna-excised female); (3) one antenna-excised male and one antenna-excised female; and (4) one intact male and one intact female. The experiment was a randomized complete block (i.e., time) design as the previous experiment. Each treatment was repeated 35 times in the end. Antennae were excised by a lance from the base of the antennae. Beetles with antennae excised were individually kept at 25 ± 1 °C, 70 ± 5% RH, 12 L: 12 D, and those can crawl normally after 24 h were used in the experiment. Each beetle pair was monitored for 2 h or until the completion of the first copulation.

Morphology of antenna by SEM

Since results from the preceding experiment indicated that antennae played an important role in mating (see Results), SEM was conducted to investigate the types of antennal sensilla in antennae. Antennae of both sexes were excised by a lance at the base and kept in a solution of acetone (50% in distilled water, Sinopharm Chemical Reagent Co., Ltd., Shanghai, China). Excised antennae were ultrasonically cleaned in acetone (50%) for 2–4 min (KM-410D Ultrasonic Cleaner; Guangzhou Kejiemeng Co., Ltd., Guangzhou, China). The antennae were then sequentially rinsed in 50 ml ethanol at concentrations of 50, 70, 80, 90 and 100% respectively for 15 min at each concentration. They were subsequently bathed in 50 ml 100% ethanol for 30 min at room temperature before being air-dried, mounted, and gold-coated with an ion sputter (SCD005; BAL-TEC, Finland) and were examined with a LEO-1530 Scanning Electronic Microscope (LEO, Germany). Types of antennal sensilla and density (number per segment) in antennal segments were identified and counted. Identification of antennal sensilla was based mainly on Zacharuk (Zacharuk, 1985).

Role of antennal segments in mating

Because SEM revealed differences in distribution and density of different types of antennal sensilla among antennal parts/segments, and between sexes, a second excision experiment examining roles of antennal segments in mating was conducted. The selection of antennal segments for testing was based on the morphology of external features of sensilla by SEM (see Table 1 for the 11 treatments). The experiment was a randomized complete block (i.e., time) design with 35 replications/treatment. The experimental procedure followed the ones previously described.

Statistical analysis

A critical value of $\alpha = 0.05$ was used for all analyses. Proportions of mating pairs under light and dark conditions, between treatments in the two antennal excision experiments were analyzed by Chi-square tests. Length, width, and distribution of antennal sensilla among antennal segments were analyzed by ANOVAs, separately for each sex. Multiple comparisons among treatments were performed using Tukey's HSD. Length, width, and distribution of antennal sensilla between sexes were analyzed by independent sample *t*-tests, separately for each antennal segment. Data on density of uniporous sensilla chaetica on 6th segment between sexes were analyzed using Mann-Whitney tests due to heteroscedasticity. All statistical analyses were performed in SPSS 15.0 for Windows (SPSS Inc.: Chicago, IL, USA). All the figures were produced in SigmaPlot 11.0 (Systat Software Inc., San Jose, California).

Results

Mating behavior

A total of 782 mating bouts were successful. The mating could be broadly grouped into two types. In the first type, a male and a female crawled in the same direction. After catching the female, the male tapped the elytra of the female with antennae and continued to climb

Table 1
Treatments in the antennal segment excision experiment.

Group	Treatment	The opposite sex
1	Intact body (♀)	Intact body (♂)
2	The ninth flagellomere were cut (♀)	Intact body (♂)
3	The sixth to ninth flagellomeres were cut (♀)	Intact body (♂)
4	The fifth to ninth flagellomeres were cut (♀)	Intact body (♂)
5	The whole flagella were cut (♀)	Intact body (♂)
6	All antennae were cut (♀)	Intact body (♂)
7	The ninth flagellomere were cut (♂)	Intact body (♀)
8	The sixth to ninth flagellomeres were cut (♂)	Intact body (♀)
9	The fifth to ninth flagellomeres were cut (♂)	Intact body (♀)
10	The whole flagella were cut (♂)	Intact body (♀)
11	All antennae were cut (♂)	Intact body (♀)